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EXAMINER
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MUMMERT, STEPHANIE KANE

ART UNIT	PAPER NUMBER
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1637

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10/26/2010

PAPER

**Please find below and/or attached an Office communication concerning this application or proceeding.**

The time period for reply, if any, is set in the attached communication.

<b>Office Action Summary</b>	<b>Application No.</b> 10/669,976	<b>Applicant(s)</b> ENGEL ET AL.	
	<b>Examiner</b> STEPHANIE K. MUMMERT	<b>Art Unit</b> 1637	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

### Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

### Status

- 1) ☒ Responsive to communication(s) filed on 23 July 2010.
- 2a) ☐ This action is **FINAL**.                      2b) ☒ This action is non-final.
- 3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

### Disposition of Claims

- 4) ☒ Claim(s) 1-2, 4-22 and 25-37 is/are pending in the application.
- 4a) Of the above claim(s) 17-22 is/are withdrawn from consideration.
- 5) ☐ Claim(s) \_\_\_\_\_ is/are allowed.
- 6) ☒ Claim(s) 1,2,4-16 and 25-37 is/are rejected.
- 7) ☐ Claim(s) \_\_\_\_\_ is/are objected to.
- 8) ☐ Claim(s) \_\_\_\_\_ are subject to restriction and/or election requirement.

### Application Papers

- 9) ☐ The specification is objected to by the Examiner.
- 10) ☐ The drawing(s) filed on \_\_\_\_\_ is/are: a) ☐ accepted or b) ☐ objected to by the Examiner.  
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).  
Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).
- 11) ☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

### Priority under 35 U.S.C. § 119

- 12) ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
- a) ☐ All    b) ☐ Some \*    c) ☐ None of:
1. ☐ Certified copies of the priority documents have been received.
  2. ☐ Certified copies of the priority documents have been received in Application No. \_\_\_\_\_.
  3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

\* See the attached detailed Office action for a list of the certified copies not received.

### Attachment(s)

- |   |   |
|---|---|
| 1) <input checked="" type="checkbox"/> Notice of References Cited (PTO-892)         | 4) <input type="checkbox"/> Interview Summary (PTO-413)           |
| 2) <input type="checkbox"/> Notice of Draftperson's Patent Drawing Review (PTO-948) | Paper No(s)/Mail Date. _____                                      |
| 3) <input type="checkbox"/> Information Disclosure Statement(s) (PTO/SB/08)         | 5) <input type="checkbox"/> Notice of Informal Patent Application |
| Paper No(s)/Mail Date _____   | 6) <input type="checkbox"/> Other: _____                          |

## **DETAILED ACTION**

### ***Continued Examination Under 37 CFR 1.114***

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on July 23, 2010 has been entered.

Applicant's amendment filed on July 23, 2009 is acknowledged and has been entered. Claim 3 and 23-24 have been canceled. Claim 1 has been amended. Claims 35-37 have been added. Claims 1-2, 4-16 and 25-37 are pending. Claims 17-22 are withdrawn from consideration as being drawn to a non-elected invention.

All of the amendments and arguments have been thoroughly reviewed and considered but are not found persuasive for the reasons discussed below. Any rejection not reiterated in this action has been withdrawn as being obviated by the amendment of the claims. The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

Claims 1-2, 4-16 and 25-37 are discussed in this Office action.

**This action is made NON-FINAL to address New Grounds of Rejection.**

## **New Grounds of Rejection**

### ***Claim Rejections - 35 USC § 103***

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

1. Claims 30-37 are rejected under 35 U.S.C. 103(a) as being unpatentable over Backus et al. (US Patent 5,705,366; January 1998) in view of Bustin, SA (Journal of Molecular Endocrinology, 2000, vol. 25, p. 169-193) and Birch et al. (US Patent 5,773,258; June 1998) as applied to claims 1-2, 4-11, 16 and 28-29 above and further in view of Henegariu et al. (Biotechniques, 1997, vol. 23, p. 504-511). Backus discloses a method of amplification of multiple target nucleic acids in the presence of a nonionic, polymeric volume exclusion agent (Abstract). Backus in view of Birch and Bustin render obvious the limitations of claims 1-2, 4-11, 16 and 28-29. However, neither Backus, Birch nor Bustin teach the simultaneous amplification of six or eight targets or more.

With regard to claim 30, Henegariu teaches an embodiment of claim 1, wherein the method is capable of coamplifying six (6) or eight (8) different target nucleic acids (Table 1, where primer set includes 9 sets of primers, amplifying 9 targets).

With regard to claim 32-33, Henegariu teaches an embodiment of claim 1, comprising six (6) or eight (8) different target nucleic acids (Table 1, where primer set includes 9 sets of primers, amplifying 9 targets).

With regard to claim 34, Henegariu teaches an embodiment of claim 1, comprising eight (8) sets of primers (Table 1, where primer set includes 9 sets of primers, amplifying 9 targets).

With regard to claim 35, Henegariu teaches an embodiment of claim 1 comprising seven (7) different target nucleic acids (Table 1, where primer set includes 9 sets of primers, amplifying 9 targets).

With regard to claim 36, Henegariu teaches an embodiment of claim 1 wherein the sets of primers are present in equimolar primer concentrations (p. 506, col. 1, where the primers are used in equimolar concentrations).

With regard to claim 37, Henegariu teaches an embodiment of claim 1 wherein a set of primers is present in a concentration that makes it a rate limiting reactant (p. 508, col. 1, where the concentration of the individual primers are varied so that one primer set is the least concentrated and so comprises a rate limiting reactant).

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have adjusted the teachings of Backus, Bustin and Birch to include the level of multiplex as taught by Henegariu to arrive at the claimed invention with a reasonable expectation for success. As taught by Henegariu, "We have examined various conditions of the multiplex PCR, using a large number of primer pairs. Especially important for a successful multiplex PCR assay are the relative concentrations of the primers at the various loci, the concentration of the PCR buffer, the cycling temperatures and the balance between the magnesium chloride and deoxynucleotide concentrations. Based on our experience, we propose a protocol for developing a multiplex PCR assay and suggest ways to overcome commonly encountered problems" (Abstract). Furthermore, regarding the issue of primer concentration,

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Henegariu teaches “Initially, equimolar primer concentrations of 0.2–0.4 mM each were used in the multiplex PCR (Figure 3c), but there was uneven amplification, with some of the products barely visible even after the reaction was optimized for the cycling conditions. Overcoming this problem required changing the proportions of various primers in the reaction, with an increase in the amount of primers for the “weak” loci and a decrease in the amount for the “strong” loci. The final concentration of the primers (0.04–0.6 mM) varied considerably among the loci and was established empirically” (p. 508, col. 1). Therefore, as Henegariu clearly teaches methods for careful adjustment and optimization of multiplex coamplification, one of ordinary skill in the art at the time the invention was made would have been motivated to have adjusted the teachings of Backus, Bustin and Birch to include the higher level of simultaneous multiplex taught by Henegariu to arrive at the claimed invention with a reasonable expectation for success.

### **Previous Grounds of Rejection**

*New claims 35 and 36 rejected in view of Grondahl.*

### ***Claim Rejections - 35 USC § 103***

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

2. Claims 1-2, 4-11, 16 and 28-29 are rejected under 35 U.S.C. 103(a) as being unpatentable over Backus et al. (US Patent 5,705,366; January 1998) in view of Bustin, SA (Journal of

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Molecular Endocrinology, 2000, vol. 25, p. 169-193) and further in view of Birch et al. (US Patent 5,773,258; June 1998). Backus discloses a method of amplification of multiple target nucleic acids in the presence of a nonionic, polymeric volume exclusion agent (Abstract).

With regard to claim 1, Backus teaches a method for the coamplification of two or more target nucleic acids having different sequence compositions, said method comprising at least 15 primary amplification cycles (col. 2, line 67 to col. 3, line 3), each amplification cycle comprising the sequential steps of:

(A) heating a reaction mixture comprising two or more target nucleic acids and a hot start DNA polymerase, or their primer extension products, at a first temperature, T1, for denaturation of the strands of the target nucleic acids or their primer extension products (col. 3, lines 4-8; col. 7, lines 11-19, where the inclusion of a polymerase and the inclusion of an antibody specific to the DNA polymerase which inhibits enzymatic activity below about 50°C and which is inactivated at higher temperatures), and

(B) priming the denatured strands with a set of unmodified or modified primers specific to and hybridizable with opposing strands of each target nucleic acid to be amplified, by cooling to a second temperature, T2 (col. 3, lines 9-12), and

(C) either as a continuation of step (B) or in a separate step, forming primer extension products in a reaction mixture of PCR reagents, by incubation at a third temperature, T3, provided that when priming and primer extension product formation are carried out in the same step, T2 and T3 are the same (col. 3, lines 13-18),

(D) in the course of the reaction in each amplification cycle or in an amplification cycle after the last primary amplification cycle, detecting two or more of the primer extension products having

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different sequence composition as an indication of coamplification of the target nucleic acids (col. 3, lines 46-48); an improvement comprising using the DNA polymerase included in the reaction mixture of step (A), a modified thermostable hot start DNA polymerase enzyme that becomes active only after incubation at temperatures above 50°C (col. 3, lines 4-8; col. 7, lines 11-19, where the inclusion of a polymerase and the inclusion of an antibody specific to the DNA polymerase which inhibits enzymatic activity below about 50°C and which is inactivated at higher temperatures), and using in at least one of the primary amplification cycles, 1 to 20 weight % of a nonionic polymeric volume exclusion agent (col. 3, lines 19-21, where the disclosed percentage of 4 weight % falls within the range(s) claimed).

With regard to claim 2, Backus teaches an embodiment of claim 1, wherein the amount of polymeric volume exclusion agent in said reaction mixture is 1 to 15 weight % (col. 3, lines 19-21, where the disclosed percentage of 4 weight % falls within the range(s) claimed).

With regard to claim 4, Backus teaches an embodiment of claim 1, wherein the amount of polymeric volume exclusion agent in said reaction mixture is 1 to 8 weight % (col. 3, lines 19-21, where the disclosed percentage of 4 weight % falls within the range(s) claimed).

With regard to claim 5, Backus teaches a method according to one of claims 1, 2 or 4, characterized in that the volume exclusion agent is selected from the group consisting of a polyether, a reaction product of a sugar with epichlorohydrin, a polysaccharide, and a polyacrylate (col. 7, lines 36-41).

With regard to claim 6, Backus teaches a method according to claim 5, characterized in that the volume exclusion agent is selected from the group of polyethers of the general formula;





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wherein R is an alkylene bridge of 1 to 6 carbon atoms - branched or unbranched - and n is an integer of 15 to 1000 (col. 3, lines 42-48).

With regard to claim 7, Backus teaches an embodiment of claim 6, characterized in that R may represent 1,2-ethylene, 1,3-propylene, 1,2-propylene, 2-hydroxy-1,3-propylene, 3-hydroxy-1,2-propylene, 1,4-butylene, 1,3-butylene, or 1,2-hexylene (col. 7, lines 48-52).

With regard to claim 8, Backus teaches an embodiment of claim 6, characterized in that the polyether is poly(ethylene glycol) (col. 7, lines 53-56, where it is noted that a preferred R group is polyethylene glycol).

With regard to claim 9, Backus teaches an embodiment of claim 8, characterized in that the polyethylene glycol has a molecular weight in the range of 1000 daltons to 2,000,000 daltons (col. 7, lines 59-67, where it is noted that the preferred range is between about 1000 to about 20,000, wherein the term about represents  $\pm 10\%$  and wherein the preferred range of molecular weights falls within the range as claimed, presuming that it is intended for the claimed ranges to be measured in daltons; col. 15, lines 19-21, where PEG-8000, a polyethylene glycol with a molecular weight of 8000 daltons was used in the examples).

With regard to claim 10, Backus teaches an embodiment of claim 8, characterized in that the poly(ethylene glycol) has a molecular weight in the range of 3000 daltons to 500,000 daltons (col. 7, lines 59-67, where it is noted that the preferred range is between about 1000 to about 20,000, wherein the term about represents  $\pm 10\%$  and wherein the preferred range of molecular weights falls within the range as claimed, presuming that it is intended for the claimed ranges to be measured in daltons; col. 15, lines 19-21, where PEG-8000, a polyethylene glycol with a molecular weight of 8000 daltons was used in the examples).

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With regard to claim 11, Backus teaches an embodiment of claim 8, characterized in that the poly(ethylene glycol) has a molecular weight of about 8000 daltons (col. 7, lines 59-67, where it is noted that the preferred range is between about 1000 to about 20,000, wherein the term about represents +/-10% and wherein the preferred range of molecular weights falls within the range as claimed, presuming that it is intended for the claimed ranges to be measured in Daltons; col. 15, lines 19-21, where PEG-8000, a polyethylene glycol with a molecular weight of 8000 daltons was used in the examples).

With regard to claim 16, Backus teaches an embodiment of claim 5, characterized in that the polyacrylate is selected from the group consisting of poly(hydroxyethyl acrylate) or poly(2,3-dihydroxypropyl acrylate) (col. 8, lines 12-15).

With regard to claim 29, Backus teaches an embodiment of claim 1 wherein each set of primers hybridizable with opposing strands of each target nucleic acid to be amplified is different (col. 5, lines 63-67, where the primers are disclosed as hybridizable with opposing strands of the target, see also col. 13, lines 29-38, where sequences of primers are provided for amplification of HIV and beta globin and the primers are each different).

Regarding claims 1, 2 and 4, while Backus teaches a reversibly modified thermostable DNA polymerase, Backus does not teach a modification that comprises a chemical modification as established in the specification. Birch teaches the reversible modification of DNA polymerase by an inhibiting agent (Abstract).

With regard to claims 1, 2 and 4, Birch teaches a chemically-modified thermostable hot start DNA polymerase enzyme that becomes active only after incubation at temperatures above 50°C (Abstract; col. 4, lines 49-58, where the reversibly inactivated enzyme is a thermostable

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DNA polymerase; col. 3, lines 1-19, where a DNA polymerase is reversibly inactivated using treatment with a modifier reagent and becomes active at a temperature of about 50 °C, col. 3, lines 44-51).

Furthermore, regarding claims 1, 2 and 4, neither Backus nor Birch explicitly teach that the two or more target nucleic acids are present at comparable copy numbers whereas the maximum difference between the lowest and the highest copy number is 10 fold. Regarding claims 2 and 4, Backus does not teach that the reaction mixture comprises a sequence specific probe which binds within the primer binding regions and which generates a fluorescent signal after hybridization.

Bustin teaches an overview of the quantitation of mRNA using a variety of methods, including quantitative real-time RT-PCR, a method which incorporates a variety of means of detection, including hybridization probes (Abstract).

With regard to claims 1-2 and 4, Bustin teaches the coamplification of nucleic acids which are present at comparable copy numbers, wherein the maximum difference between the lowest and highest copy number is 10-fold (p. 182, col. 1, 2<sup>nd</sup> paragraph, where the normalization of quantification of a target nucleic acid is accomplished through co-amplification of an internal control target sequence, referred to as an endogenous control. It is also noted that the endogenous control should be expressed at roughly the same level as the RNA under study; see also p. 185, 'multiplex RT-PCR' heading, where multiple primer sets are used to amplify multiple specific targets simultaneously).

With regard to claim 1, Bustin discloses a sequence specific probe which binds within the primer binding regions and which generates a fluorescent signal after hybridization (Figure 3 and

p. 174, where molecular beacon probes were described; Figure 4A-C right side and p. 177, where ‘hybdrization probes’ were described; Figure 5, p. 177, where TaqMan hydrolysis probes were described).

With regard to claim 28, Bustin teaches an embodiment of claim 1, characterized in that the sequence specific labeled probe is fluorescently labeled (Figure 3 and p. 174, where molecular beacon probes were described and are fluorescently labeled; Figure 4A-C right side and p. 177, where ‘hybdrization probes’ were described; Figure 5, p. 177, where TaqMan hydrolysis probes were described).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to have extended the teachings of Backus to incorporate the fluorescent hybridization probes and coamplification of a target sequence of comparable copy number of Bustin, to arrive at the claimed invention with a reasonable expectation for success. As taught by Bustin, “The accepted method for minimizing these errors and correcting for sample-to-sample variation is to amplify, simultaneously with the target, a cellular RNA that serves as an internal reference against which other RNA values can be normalized. The ideal internal standard should be expressed at a constant level among different tissues of an organism, at all stages of development, and should be unaffected by the experimental treatment. In addition, an exogenous control should also be expressed at roughly the same level as the RNA under study” (p. 182, col. 1). Therefore, one of ordinary skill in the art at the time the invention was made would have been motivated to extend the coamplification in the presence of a polymeric volume exclusion agent taught by Backus to incorporate the teachings of Bustin with a reasonable expectation for

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success in order to achieve quantitative coamplification of two nucleic acid targets of similar copy number.

Furthermore, it would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to have extended the teachings of Bustin and Backus to include the chemical modification through reaction with an chemical as taught by Birch to arrive at the claimed invention with a reasonable expectation for success. The reversible modification taught by Backus includes “an antibody specific to the DNA polymerase, which antibody inhibits enzymatic activity at a temperature below about 50°C, but which antibody is deactivated at higher temperatures” (col. 7, lines 11-20). In a related technique, Birch teaches methods that “use a reversibly inactivated thermostable enzyme which can be reactivated by incubation in the amplification reaction mixture at an elevated temperature” (col. 2, lines 62-65). Birch also teaches a preferred embodiment wherein “the amplification reaction is a polymerase chain reaction (PCR) and a reversibly-inactivated thermostable DNA polymerase is used. The reaction mixture is incubated prior to carrying out the amplification reaction at a temperature which is higher than the annealing temperature of the amplification reaction. Thus, the DNA polymerase is inactivated until the temperature is above the temperature which insures specificity of the amplification reaction, thereby reducing non-specific amplification” (col. 4, lines 49-58). Both Backus and Birch teach modification that is reversible with an increase in temperature. Therefore, one of ordinary skill in the art at the time the invention was made would have been motivated to have extended the teachings of Bustin and Backus to include the chemical modification through reaction with a chemical as taught by Birch to arrive at the claimed invention with a reasonable expectation for success.

3. Claims 12-15 are rejected under 35 U.S.C. 103(a) as being unpatentable over Backus et al. (US Patent 5,705,366; January 1998) in view of Bustin, SA (Journal of Molecular Endocrinology, 2000, vol. 25, p. 169-193) and Birch et al. (US Patent 5,773,258; June 1998) as applies to claims 1-2, 4-11, 16 and 28-29 and further in view of Reed et al. (US Patent 5,459,038; October 1995) and Demke et al. (Biotechniques, 1992, vol. 12, no. 3, p. 333-334). Backus discloses a method of amplification of multiple target nucleic acids in the presence of a nonionic, polymeric volume exclusion agent (Abstract).

Regarding claims 12-15, while Backus teaches amplification in the presence of Dextran sulfate as inhibiting to amplification, Demke provides an explanation that while dextran sulfate is inhibitory to PCR amplification, Dextran does not inhibit amplification via PCR.

Demke does not provide explicit teaching that dextran provides an improvement to PCR amplification without the inclusion of a volume exclusion agent. Reed teaches amplification of samples in the presence of Dextran (col. 19, lines 9-26, where the inclusion of Dextran results in more efficient amplification).

With regard to claim 12, Reed teaches an embodiment of claim 5, characterized in that the volume exclusion reagent is a dextran (col. 19, lines 9-26, where the inclusion of Dextran results in more efficient amplification).

With regard to claim 13, Reed teaches an embodiment of claim 12, characterized in that the dextran has a molecular weight in the range of 1000 to 2,000,000 (col. 16, lines 10-20, where the dextran is T500, which comprises a MW of 500,000).

With regard to claim 14, Reed teaches an embodiment of claim 12, characterized in that the dextran has a molecular weight in the range of 3000 to 500,000 (col. 16, lines 10-20, where the dextran is T500, which comprises a MW of 500,000).

With regard to claim 15, Reed teaches an embodiment of claim 12, characterized in that the dextran has a molecular weight in the range of 40,000 daltons to 60,000 daltons (col. 16, lines 10-20, where the dextran is T500, which comprises a MW of 500,000, see obviousness rejection below).

While Reed teaches a Dextran of molecular weight 500,000, Reed also teaches that dextran generally provides an improvement over PCR amplification reactions that are not conducted in the presence of dextran. Thus, an ordinary practitioner would have recognized that the results optimizable variables of time, concentration and product amount could be adjusted to maximize the desired results. As noted in *In re Aller*, 105 USPQ 233 at 235,

More particularly, where the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation.

Routine optimization is not considered inventive and no evidence has been presented that the results were other than routine, that the products resulting from the optimization have any unexpected properties, or that the results should be considered unexpected in any way as compared to the closest prior art.

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to have extended the teachings of Backus to incorporate the fluorescent hybridization probes and coamplification of a target sequence of comparable copy number the of Bustin, to arrive at the claimed invention with a reasonable expectation for success. As taught by

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Bustin, “The accepted method for minimizing these errors and correcting for sample-to-sample variation is to amplify, simultaneously with the target, a cellular RNA that serves as an internal reference against which other RNA values can be normalized. The ideal internal standard should be expressed at a constant level among different tissues of an organism, at all stages of development, and should be unaffected by the experimental treatment. In addition, an exogenous control should also be expressed at roughly the same level as the RNA under study” (p. 182, col. 1). Therefore, one of ordinary skill in the art at the time the invention was made would have been motivated to extend the coamplification in the presence of a polymeric volume exclusion agent taught by Backus to incorporate the teachings of Bustin with a reasonable expectation for success in order to achieve quantitative coamplification of two nucleic acid targets of similar copy number.

Furthermore, it would have been *prima facie* obvious in view of the teachings of Demke and Reed to include dextran into the method of amplification taught by Backus in view of Bustin. First, it is noted that Backus teaches amplification in the presence of PEG and dextran sulfate. While Backus teaches that dextran sulfate is inhibitory to amplification, Demke teaches “the inhibitory nature of some polysaccharides with free acidic groups is further demonstrated by contrasting dextran and dextran sulfate. Dextran (neutral) has no interfering effects at 500:1 ratio, whereas dextran sulfate was very inhibitory (Table 1). Therefore, considering the teachings of Demke, it would have been *prima facie* obvious to substitute the dextran sulfate taught by Backus for the equivalent dextran as taught by Demke. Furthermore, as taught by Reed, “the inclusion of polysaccharide dextran (or similar) results in three unique advantages: firstly, its inclusion results in more efficient amplification leading to markedly higher sensitivity and



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specificity” (col. 19, lines 9-26). Therefore, it would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to have extended the method taught by Backus to include dextran as taught by Reed and Demke to achieve efficient amplification with higher sensitivity and specificity with a reasonable expectation for success.

4. Claim 25 is rejected under 35 U.S.C. 103(a) as being unpatentable over Backus et al. (US Patent 5,705,366; January 1998) in view of Bustin, SA (Journal of Molecular Endocrinology, 2000, vol. 25, p. 169-193) and Birch et al. (US Patent 5,773,258; June 1998) as applied to claims 1-2, 4-11, 16 and 28-29 above, and further in view of Ivanov et al. (US Patent 6,183,998; February 2001). Backus discloses a method of amplification of multiple target nucleic acids in the presence of a nonionic, polymeric volume exclusion agent (Abstract).

Backus in view of Bustin and Birch renders obvious claims 1-2, 4-11, 16 and 28 as recited in the 103 rejection stated above. While Backus in view of Bustin and Birch teaches a chemically modified DNA polymerase, neither Backus, Bustin or Birch teaches that the modification is due to a reaction with an aldehyde. Ivanov teaches reversible modification of DNA polymerases through reaction with an aldehyde (Abstract).

With regard to claim 25, Ivanov teaches an embodiment of claim 1, 2 or 4, wherein said chemically modified DNA polymerase is modified by reaction with an aldehyde (Abstract).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to have extended the teachings of Bustin and Backus to include the chemical modification through reaction with an aldehyde as taught by Ivanov to arrive at the claimed invention with a reasonable expectation for success. The reversible modification taught by

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Backus includes “an antibody specific to the DNA polymerase, which antibody inhibits enzymatic activity at a temperature below about 50°C, but which antibody is deactivated at higher temperatures” (col. 7, lines 11-20). In a related technique, Ivanov teaches “for reversible inactivation of thermostable enzymes using a chemical modification under essentially aqueous conditions. In particular, the thermostable enzymes of the present invention are reversibly modified in the presence of an aldehyde”. Furthermore, Ivanov teaches “enzymatic activity of the present chemically modified enzymes is increased at least two-fold within thirty minutes when incubated at a more elevated temperature, i.e. above 50 °C, preferably at a temperature of 75 °C to 100 °C” (col. 3, lines 1-14). Both Backus and Ivanov teach modification that is reversible with an increase in temperature. Therefore, one of ordinary skill in the art at the time the invention was made would have been motivated to have extended the teachings of Bustin and Backus to include the chemical modification through reaction with an aldehyde as taught by Ivanov to arrive at the claimed invention with a reasonable expectation for success.

5. Claims 26-27 are rejected under 35 U.S.C. 103(a) as being unpatentable over Backus et al. (US Patent 5,705,366; January 1998) in view of Bustin, SA (Journal of Molecular Endocrinology, 2000, vol. 25, p. 169-193) and Birch et al. (US Patent 5,773,258; June 1998) as applied to claims 1-2, 4-11, 16 and 28-29 above and further in view of Mansfield et al. (Molecular Cellular Probes, 1995, vol. 9, p. 145-156). Backus discloses a method of amplification of multiple target nucleic acids in the presence of a nonionic, polymeric volume exclusion agent (Abstract).

6. Backus in view of Bustin and Birch render obvious all of the limitations of claims 1-2, 4-11, 16 and 28. However, neither Backus, Bustin or Birch teach that one of the primers is

fluorescently labeled or that one of the primers is labeled with a specific binding moiety.

Mansfield teaches a variety of primer labeling techniques (Abstract).

With regard to claim 26, Mansfield teaches an embodiment of claim 1, characterized in that one of the primers of each primer set is fluorescently labeled (p. 145, col 2; p. 146, col. 1, where PCR incorporating a 5' end tagged primer sequence labeled with a fluorescent nucleotide is described).

With regard to claim 27, Mansfield teaches an embodiment of claim 1, characterized in that one of the primers of each primer set is labeled with a specific binding moiety (p. 145, col 2, where a small molecule like fluorescein, biotin or digoxigenin can be incorporated into the detection reagent; Figure 1, where the biotin label is attached to a probe, but the attachment of a probe or a primer is equivalent).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to have adjusted the teachings of Backus, Bustin and Birch to include the labeled primers of Mansfield to arrive at the claimed invention with a reasonable expectation for success. As taught by Mansfield, "fluorescent, chemiluminescent, bioluminescent and colorimetric approaches have been used as alternatives to radioactive detection methods. Fluorogenic or chemiluminescent or chemiluminescent substrates allow subattomole level detection of DNA labels in solid-phase membrane-based hybridization assays with much greater sensitivity than using colorimetric methods". Mansfield also emphasizes that "fluorescence detection is by far the most versatile" (p. 154, col. 1). Therefore, one of ordinary skill in the art at the time the invention was made would have been motivated to have adjusted the teachings of

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Backus, Bustin and Birch to include the labeled primers of Mansfield to achieve sensitive detection of amplification products.

7. Claims 30-36 are rejected under 35 U.S.C. 103(a) as being unpatentable over Backus et al. (US Patent 5,705,366; January 1998) in view of Bustin, SA (Journal of Molecular Endocrinology, 2000, vol. 25, p. 169-193) and Birch et al. (US Patent 5,773,258; June 1998) as applied to claims 1-2, 4-11, 16 and 28-29 above and further in view of Grondahl et al. (J. Clin. Micro., 1999, 37(1):1-7) . Backus discloses a method of amplification of multiple target nucleic acids in the presence of a nonionic, polymeric volume exclusion agent (Abstract). Backus in view of Birch and Bustin render obvious the limitations of claims 1-2, 4-11, 16 and 28-29. However, neither Backus, Birch nor Bustin teach the simultaneous amplification of six or eight targets or more.

With regard to claim 30, Grondahl teaches an embodiment of claim 1, wherein the method is capable of coamplifying six (6) or eight (8) different target nucleic acids (p. 2, col. 1, where the primers for the nine different target nucleic acids are provided; Further, see abstract and title, where it is clear that multiplex amplification is carried out in a single tube and so at least nine targets are coamplified).

With regard to claim 32-33, Grondahl teaches an embodiment of claim 1, comprising six (6) or eight (8) different target nucleic acids (p. 2, col. 1, where the primers for the nine different target nucleic acids are provided; Further, see abstract and title, where it is clear that multiplex amplification is carried out in a single tube and so at least nine targets are coamplified).

With regard to claim 34, Grondahl teaches an embodiment of claim 1, comprising eight (8) sets of primers (p. 2, col. 1, where the primer sets for the nine different target nucleic acids are listed).

With regard to claim 35, Grondahl teaches an embodiment of claim 1 comprising seven (7) different target nucleic acids (p. 2, col. 1, where the primers for the nine different target nucleic acids are provided; Further, see abstract and title, where it is clear that multiplex amplification is carried out in a single tube and so at least nine targets are coamplified).

With regard to claim 36, Grondahl teaches an embodiment of claim 1 wherein the sets of primers are present in equimolar primer concentrations (p. 2, col. 2, where the primers are used in equimolar concentrations, 1uM each primer).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to have adjusted the teachings of Backus, Bustin and Birch to include the level of multiplex as taught by Grondahl to arrive at the claimed invention with a reasonable expectation for success. As taught by Grondahl, “A multiplex reverse transcription-PCR (RT-PCR) assay was developed to allow in one test the detection of nine different microorganisms (enterovirus, influenza A and B viruses, respiratory syncytial virus [RSV], parainfluenzaviruses type 1 and type 3, adenovirus, *Mycoplasma pneumoniae*, and *Chlamydia pneumoniae*) that do not usually colonize the respiratory tracts of humans but, if present, must be assumed to be the cause of respiratory disease.” (Abstract). This method is designed to overcome “the disadvantage of requiring different and time-consuming assay conditions for each organism detected and the use of several tubes for one sample, thus enlarging the risk of cross contamination” (p. 1, col. 2). Therefore, as Grondahl clearly teaches an improvement provided

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by multiplex coamplification carried out in a single tube, one of ordinary skill in the art at the time the invention was made would have been motivated to have adjusted the teachings of Backus, Bustin and Birch to include the higher level of simultaneous multiplex taught by Grondahl to arrive at the claimed invention with a reasonable expectation for success.

### **Response to Arguments**

Applicant's arguments filed July 23, 2010 have been fully considered but they are not persuasive.

Applicant traverses the rejection of claims 1-2, 4-11, 16, 23 and 24 under Backus in view of Bustin and Birch. Applicant traverses on the grounds that "the presently claimed methods provide results that are unexpectedly superior to those that could have been reasonably expected". Applicant summarizes the teachings of Markoulatos as suggesting that "'development of an efficient multiplex PCR usually requires strategic planning and multiple attempts to optimize reaction conditions" (p. 9 of remarks). Applicant points to Bustin as teaching that it is best to limit multiplexed targets "if quantification is the main aim" (p. 9 of remarks). Applicant concludes that based on the combination of references, one of skill "would have believed that a fair amount of luck and an enormous amount of optimization over a long and incomplete list of variables, conditions and variables would be required in order to develop a new multiplex PCR assay" (p. 10 of remarks). Applicant goes on to argue that "the lack of a need for optimization that renders the results obtained by the claimed method unexpectedly superior" (p. 10 of remarks).

Next, Applicant argues the role of unexpected results associated with the instant method, citing to Exhibit A, New QIAGEN Multiplex PCR Kit, QIAGEN News 5:13-16, November 2002. Applicant points to a diagram in the reference that indicates that there is no optimization and to results in Figure 1. Applicant emphasizes that "only the reactions using the Applicant's standard multiplex PCR Master Mix containing Q solution were successful in simultaneously amplifying the 16 target nucleic acid targets" and that the "Q solution' contains a polymeric volume exclusion agent as disclosed". Applicant emphasizes again that one of ordinary skill would view the optimization of multiplex amplification as "potentially endless trial and error experimentation... with no guaranty and no reasonable expectation of arriving at results similar to what is shown" (p. 12 of remarks).

Next, Applicant points to Groendahl as substantiating the unexpected nature of the instant method. Applicant argues again that one of skill "would have thought that the authors in Groendahl needed to carry out extensive optimization experiments in order to establish the described protocol" in order to avoid "accumulation of by-products from unspecific annealing" (p. 13 of remarks). Applicant argues that Groendahl does not provide any advances in the field of multiplex.

Finally, Applicant concludes that "the claimed methods demonstrate a superior advantage that the person of ordinary skill... would have found surprising or unexpected" and that these "unexpectedly superior results are sufficient rebut any prima facie case of obviousness". Applicant also points to commercial success of products which include the multiplex amplification kit as described.

These arguments have been carefully considered, but these arguments are not persuasive. First, while Applicant's arguments regarding the "design and execution of multiplex polymerase chain reactions" and that it is "anything but a straightforward undertaking" (p. 10 of remarks) are noted, the argument that the process of multiplex amplification of multiple targets is complex, or even that the process requires optimization does not provide evidence of a lack of reasonable expectation for success. While citation to additional references which highlight the elements that must be optimized for successful multiplex amplification are appreciated, again it is noted that Applicant is effectively arguing that multiplex amplification requires optimization. A need for optimization of a multiplex amplification is not unexpected to one of ordinary skill and does not provide any basis for a lack of reasonable expectation for success. Further, while Applicant points to multiple references used in the rejection, including Bustin and Groendahl, as evidence that the method is unexpected because it does not require substantial optimization and one of skill would be discouraged or turned away from the process of multiplex amplification because of a lack of expectation of success, one of ordinary skill would understand the factors that need to be adjusted to optimize any type of multiplex amplification. There are multiple references, such as Henegariu newly cited above, which clearly lay out the various factors that are key to successful multiplex amplification and a methodical process for evaluating and adjusting these factors. Therefore, in light of this, Applicant's arguments regarding the "daunting" task of optimization of multiplex amplification are not persuasive. Again it is reiterated that arguing that a method requires optimization is not evidence of a lack of reasonable expectation for success. A reasonable expectation of success does not require a **guarantee** of success (emphasis added).

Next, while Applicant's arguments regarding the unexpected features of the instantly claimed invention are noted, these arguments are not persuasive for a variety of reasons. Most importantly, while Applicant's arguments are noted, Applicant's attention is brought to the MPEP



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regarding this issue. First, MPEP 716.01(c) II teaches that attorney arguments cannot take the place of evidence in the record.

#### ATTORNEY ARGUMENTS CANNOT TAKE THE PLACE OF EVIDENCE

The arguments of counsel cannot take the place of evidence in the record. In re Schulze, 346 F.2d 600, 602, 145 USPQ 716, 718 (CCPA 1965). Examples of attorney statements which are not evidence and which must be supported by an appropriate affidavit or declaration include statements regarding unexpected results, commercial success, solution of a long-felt need, inoperability of the prior art, invention before the date of the reference, and allegations that the author(s) of the prior art derived the disclosed subject matter from the applicant. See MPEP § 2145 generally for case law pertinent to the consideration of applicant's rebuttal arguments.

Also, MPEP 716.02(g) provides further basis for this requirement

#### Declaration or Affidavit Form

"The reason for requiring evidence in declaration or affidavit form is to obtain the assurances that any statements or representations made are correct, as provided by 35 U.S.C. 25 and 18 U.S.C. 1001." Permitting a publication to substitute for expert testimony would circumvent the guarantees built into the statute. Ex parte Gray, 10 USPQ2d 1922, 1928 (Bd. Pat. App. & Inter. 1989). Publications may, however, be evidence of the facts in issue and should be considered to the extent that they are probative.

Next, while the content of the publication offered in Exhibit A and the results are impressive, the substance of the reference and the arguments are not necessarily persuasive in rendering the instantly claimed method as non-obvious because the data offered is not commensurate in scope with the invention as claimed. First, the copy numbers of the targets amplified in the method were not clearly taught in the reference, therefore a key feature of the instant method is not taught. Next, while Applicant argues that the Q solution comprises the volume exclusion agent as instantly claimed, there is no evidence of this teaching that is immediately apparent in the reference. Therefore, due to the presentation of the evidence in argument form and not in declaration form, the lack of specific teaching of copy number amounts and the lack of specific teaching of the volume exclusion agent amount to a conclusion that the arguments regarding unexpected results are not persuasive and the rejections are maintained.

Applicant traverses the rejection of claims 12-15 as being obvious over Backus, Bustin, Birch, Reed and Demke. Applicant argues "as set forth above and in previous argumentation,

Applicant's submit that the Office Action has not set forth a proper prima facie case of obviousness, as there would not have been a reasonable expectation of success" and that "the unexpected results described herein clearly rebut this showing" (p. 15 of remarks). These arguments have been considered, but are not persuasive for the reasons stated above. Therefore, the rejections are maintained.

Applicant traverses the rejection of claim 25 as being obvious over Backus, Bustin, Birch and Ivanov. Applicant argues "as set forth above and in previous argumentation, Applicant's submit that the Office Action has not set forth a proper prima facie case of obviousness, as there would not have been a reasonable expectation of success" and that "the unexpected results described herein clearly rebut this showing" (p. 16 of remarks). These arguments have been considered, but are not persuasive for the reasons stated above. Therefore, the rejections are maintained.

Applicant traverses the rejection of claim 26-27 as being obvious over Backus, Bustin, Birch and Mansfield. Applicant argues "as set forth above and in previous argumentation, Applicant's submit that the Office Action has not set forth a proper prima facie case of obviousness, as there would not have been a reasonable expectation of success" and that "the unexpected results described herein clearly rebut this showing" (p. 16 of remarks). These arguments have been considered, but are not persuasive for the reasons stated above. Therefore, the rejections are maintained.

### ***Conclusion***

No claims are allowed. All claims stand rejected.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to STEPHANIE K. MUMMERT whose telephone number is

(571)272-8503. The examiner can normally be reached on M-F, 9:00-5:30. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on 571-272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Stephanie K. Mummert/  
Primary Examiner, Art Unit 1637